

THE MORPHOLOGY AND DEVELOPMENT OF *ANTENNULARIA ENGLERIANA* (P. Henn.) v. Höhn.¹

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ABSTRACT

The askostroma results from the coiling and anastomosing of the superficial hyphae. Pseudoparaphyses arise as outgrowths of cells of the pseudoparenchymatous centrum and are connected to the top and bottom of the locule. Asci grow up between the pseudoparaphyses and centrum development is of the Pleospora type.

UITTREKSEL

DIE MORFOLOGIE EN ONTWIKKELING VAN *ANTENNULARIA ENGLERIANA* (P. HENN.) V. HOHN.

Die askostroma ontstaan as gevolg van die opdraai en saamgroei van die oppervlakkige swamdrade. Pseudoparafises ontwikkel as uitgroeiels van die pseudoparenchiem sentrum en is aan die bokant en onderkant van die hokkie van die askostroma geheg. Askusse groei opwaarts tussen die pseudoparafises en die ontwikkeling van die askostroma geskied soos dié van die Pleospora-tipe.

INTRODUCTION

Antennularia engleriana is a fungus which is parasitic on the stems of species of *Erica* (Ericaceae). This fungus has been assigned to various genera over the last 70 years. It was perhaps Doidge (1941) who described it most comprehensively under the name *Dimerosporiopsis engleriana*, a combination proposed by Hennings (1901). Von Höhnelt (1909) transferred it to the genus *Antennularia* whilst retaining the epithet of Hennings, hence *Antennularia engleriana* (P. Henn.) v. Höhn. In 1928, v.d. Byl proposed a new combination for this fungus, this being *Gibbera engleriana* (P. Henn.) v.d. Byl.

According to the taxonomic characteristics followed by Müller and von Arx (1962), this fungus must be placed among the Venturiaceae on spore characteristics and the presence of an hypostroma. However, the possession of superficial hyphae would preclude its inclusion in the genus *Gibbera* and the fungus has thus been transferred back to *Antennularia* under the original combination of *A. engleriana*. The use of the latter name here is one of convenience. In the light of the lack of detailed developmental studies on genera noted as synonyms for this fungus, it seems futile to debate the validity of the assignation of any particular name. This description is therefore offered purely from a developmental point of view with the object of finding out whether or

¹ Based on a dissertation submitted by one of the authors (S.K-M.T.) to Rhodes University in partial fulfilment for the degree of Doctor of Philosophy.



not the formation of the centrum conforms with one of the types proposed by Luttrell (1951) and, on this basis, to suggest its taxonomic position.

MATERIALS AND METHODS

The material for this study was found growing on the stems of *Erica*. Collections of parasitised *Erica brownleea* Bol. were made during April 1965/6 and also in March, 1966 from the Hogsback area (nr. Alice, E. Cape) and of *Erica nemorosa* Kl. ex Benth. in the Belmont Valley, Grahamstown, Cape Province, during November, 1965 and February/March, 1966.

For developmental studies, portions of cortical tissue plus fungus were fixed in formalin-acetic-alcohol, dehydrated in a butyl alcohol series, imbedded in paraffin wax (55°C.) and sectioned at 8 μ on a rocker type microtome. For certain sections, a sledge microtome was also employed.

The sections were, in most cases, stained in Heidenhain's haematoxylin and counterstained in Orange G (Johansen, 1940). Temporary squash preparations were made from fresh and fixed material. The early stages of ascocarp formation were deduced from the superficial hyphae mounted in lactophenol but for studies of the pseudoparaphyses, the preparation and staining techniques according to Wittman (1962) were employed. For the investigation of the hypertrophy of the stem, sections were cut in the transverse, radial and tangential planes and stained in safranin and fast green.

HOST-PARASITE RELATIONS

Fungal invasion is initiated on very young shoots of the host plant resulting in the hypertrophy of these areas (Fig. 1A). The peridermal areas become cracked and the entire area is covered with a brown to black mass of inter-

FIG. 1A.

Infected plants of *Erica nemorosa* showing the hypertrophied, black areas on the stems.

FIG. 1B.

Transverse section of infected part of the stem passing through ascocarps and tufts of erect hyphae.

FIG. 1C.

Tufts of erect hyphae with ascocarp initial at the tips.

FIG. 1D.

Vertical section through very young ascocarp. Ascocarp composed of compacted pseudoparenchyma.

FIG. 1E.

Pseudoparaphyses from mature ascocarp (squash preparation), composed of distinctly branched hyphae of uninucleate cells.

FIG. 1F.

Vertical section of ascocarp. Pseudoparaphyses traverse the locule from top to bottom and darker staining ascogenous hyphae grow up between these pseudoparaphyses.

FIG. 1G.

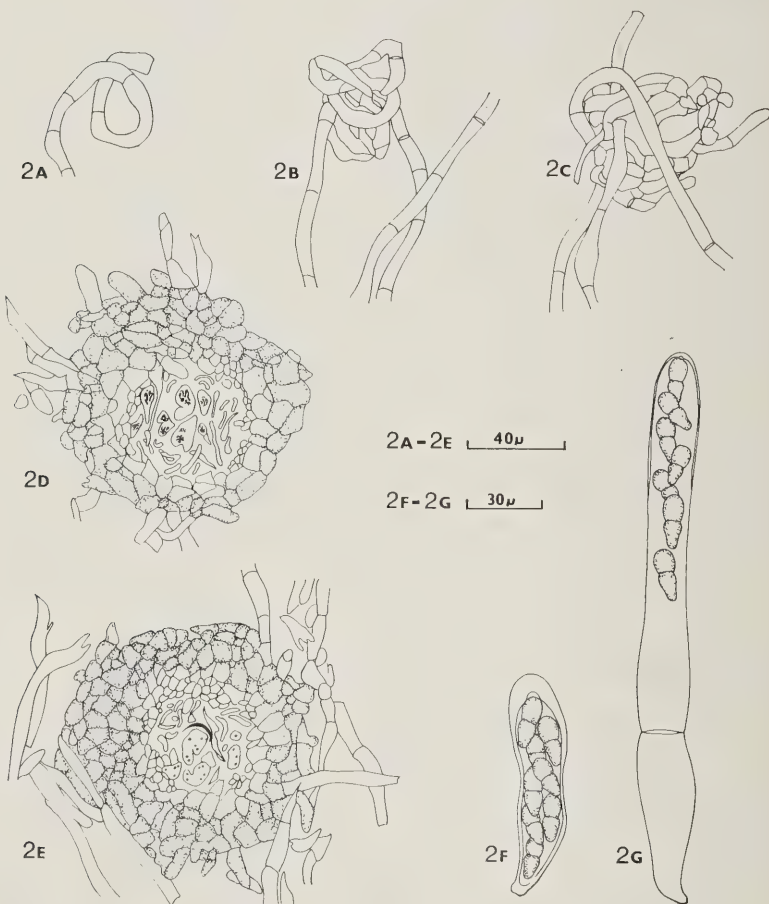
Ascogenous cells concentrated toward the base of the locule with ascal initials growing up between the pseudoparaphyses.

FIG. 1H.

Mature ascocarp with persistent pseudoparaphyses. Asci originate from the base of the locule. (Ascal walls do not show up in the photomicrograph).

twining fungal material. On the stem surfaces, prominent cushions of a cellular construction, brown in colour, are present and from these arise tufts of erect hyphae (Figs. 1B, C) which, due to the closeness of the cushions, appear to completely clothe the infected parts. Ascocarps are to be found associated with the erect hyphae (Fig. 1C).

With regard to the tissue or tissues to which the hypertrophy of the stem could be attributed, both normal, unparasitised stems were sectioned and



observed for comparison with infected stems. Hyphal growth is most extensive in the cortex of the stem. Pockets of hyphae, consisting of threads or compacted pseudoparenchyma and homologous with the cushions of tissue occurring around the stem periphery, contrast with the surrounding yellow to hyaline tissue. Interconnecting hyphae between adjacent hyphal pockets consist of multicellular threads. Such threads, made up of short, uninucleate cells, extensively traverse the primary and secondary phloem as well as most of the secondary xylem. A point of note is the fact that the hyphae are entirely intercellular.

Although cortical tissues and phloem in the infected areas are far wider than those in the uninfected zones, hypertrophy is essentially the result of an excessive production of secondary xylem. In a section through a stem which showed hypertrophy over half its circumference, with the other half uninfected, the secondary xylem was almost double the width of that in the uninfected area. The fungal strands passing through the secondary xylem are continuous and are either separate from the parenchymatous rays or else intermingled with the ray cells. Of comparatively infrequent occurrence are the presence of vertically interconnecting strands from one radial hyphal "ray" to an adjacent one. Transverse interconnecting strands in the secondary tissues have not been seen.

ASCIGEROUS LOCULE

De Bary (1887) designated the term "symphogenous" to the type of pycnidial development where young hyphal branches interwove to give rise to a compact knot of tissue, this ultimately forming the fruiting body. Such a term might equally be used to describe the initial stages in the development of the ascocarp encountered in this fungus. The erect hyphae clothing the surface of the stem in the infected area do not normally branch. However, some form short loops (Fig. 2A). It appears as if there may be some mechanism governing the formation of ascocarps for it has been observed that only the contiguous hyphae

FIGS. 2A-2C.

Stages from the looping of-, to the anastomosing of the superficial hyphae of the fungus in the formation of an ascocarp.

FIG. 2D.

Transverse section of young ascocarp showing ascogonia surrounded by pseudoparaphyses.

FIG. 2E.

Vertical section of young ascocarp. Centrum composed of multinucleate, lobed ascogonia. Pseudoparaphyses, which have originated above the ascogonia, grow downwards.

FIG. 2F.

Unextended ascus showing the thick wall and containing eight bicellular ascospores.

FIG. 2G.

Ascus after extension with single collar of the ectoascus. Ascospores have moved up toward the apex.

contribute to the formation of any specific fruiting body and that the looping of the hyphae only occur among closely applied strands. Eventually, these hyphae mass and intertwine and form a knot of compacted tissue (Figs. 2B, 2C) which, in the early stages, show a pseudoparenchymatous internal structure (Fig. 1D). In very young stages, these knots are raised well above the surface of the stem (Fig. 1C). This observation was made previously by Doidge (1941) whom, it appears, did not connect the early stages of their development with the looping and anastomosing of the erect hyphae. These ascocarps are gradually drawn downward toward the surface of the host stem and a range of intermediate stages between the very young ascocarp to the mature ones is present at any one time. In the case of the mature ascocarps, these are closely applied to the cushions of pseudoparenchyma mentioned previously (Fig. 1B). Even in this latter state, very short, intertwined hyphae supporting the ascocarps are still evident. Size increase of the ascocarps are the result of the more proximal hyphae anastomosing with the initial mass and also the division and differentiation of the pseudoparenchymatous cells making up the young ascocarp.

Initially, no fusion of the contributing hyphae takes place as seen in a section of a very young fruiting body measuring some 40μ at its largest diameter. In an older ascocarp, 65μ — 70μ in diameter, the early stages in differentiation are detectable with the tissue in the centre being decidedly pseudoparenchymatous and consisting of closely compacted, polygonally shaped cells (Fig. 1D). Cell walls of the latter also tend to be lightly staining relative to the darker walls of the outermost cells of the ascocarp. This central tissue of thin-walled cells constitutes the early development of the centrum.

Within the central pseudoparenchyma, changes in structure soon become apparent. From some of the cells, hypha-like outgrowths are produced which, by their elongation, disrupt the neat, contiguous arrangement of cells seen in the previous stage. Concomitant with this development, a number of cells, centrally placed, can be distinguished from the others. These are conspicuous by their prominent nuclei composed of clearly defined chromosomes and nucleoli (Fig. 2D). These are the reproductive initials and are lobed but the compactness of the constituent cells did not allow for distinguishing between a separate ascogonium and antheridium. Consequently, the structures are considered to constitute one or more lobed ascogonia. The surrounding thread-like outgrowths are now more distinct and can be seen to arise from some of the pseudoparenchymatous cells. These are the initials of the pseudoparaphyses (sensu Luttrell, 1965) and they already show septation into component cells. In the vertical section of the ascocarp (Fig. 2E), the outgrowths can be seen to arise from the uppermost cells of the centrum and are most prominent in the region immediately above the ascogonia. The nuclear state of the latter are better defined and their multinucleate condition clearly apparent. Some of the nuclei are

paired, suggesting a dikaryotic phase. The details of plasmogamy and karyogamy have not been elucidated.

In its further development, the ascogonium gives rise to a number of branches, the ascogenous hyphae, which extend up among the vertically orientated pseudoparaphyses (Fig. 1F). The ascogonial branches are distinctly multinucleate with dikaryons apparent here and there. The structurally distinct pseudoparaphyses consist of almost parallel-running strands of cells which traverse the locule from top to bottom, are freely branched (Fig. 1E) and extend from the pseudoparenchyma at the top of the locule and through the ascogenous hyphae to the opposite side. These pseudoparaphyses remain evident throughout the further stages of centrum development. The suggestion by some authors (Cavara & Mollica, 1907 and Arnold, 1928) that the elongation of the pseudoparaphyses is instrumental in the size increase of the fruiting structure has not been ascertained in this study. Nevertheless, it is significant that the growth in length of the pseudoparaphyses does keep pace with the general growth of the ascocarp.

As a result of the rapid elongation of the pseudoparaphyses, the ascogonial complex becomes confined to the lower half or base of the centrum (Fig. 1G) with young asci, each conspicuous by a large fusion nucleus, growing upward amongst the pseudoparaphyses. The asci line the whole base of the locule with a tendency to be more concentrated toward the periphery. Finally, in more mature ascocarps filled with well-developed asci containing ascospores (Fig. 1H), the pseudoparaphyses are still clearly evident with the asci positioned between the strands.

In dry conditions, the globose ascocarps are flattened in the ostiolar area. The apical cells of the outer, dark, thick-walled layers of each of the ascocarps gradually disintegrate to form a pore of irregular outline. The thin-walled pseudoparenchyma and pseudoparaphyses remain as a continuous layer beneath the pore even when the opening has been formed. On wetting, the ascocarp absorbs water rapidly and swells up.

From the ascogenous hyphae, asci arise by typical crozier formation. Asci are clavate (Fig. 2F) and bitunicate and contain eight spores each of which is unequally bicellular, up to 16μ in length and 5μ — 6μ in width, slightly constricted at the septum and pluriguttulate. Observations of the bitunicate effect were made on a clump of asci either squashed or dissected out of locules. After extension, the ascus exceeded the length of the original by as much as two and a half times (Fig. 2G). Often, extension was abnormal in that the ectoascus was inclined to split in two or more places with the result that a number of collars or constricting rings were evident. Such a result has also been observed by Luttrell (1951) in *Dothidea collecta* and is considered an atypical condition resulting

from extension in water. In normal extension, only a single collar or ectoascal wall remains.

On spore ejection, the spores are arranged in a single file in the upper half of the ascus and are shot out in quick succession. The orientation of each of the spores is also a consistent feature in that the shorter, blunter hemicell always points toward the apex. After all the spores have been shot out, the ascus contracts, revealing a thick endoascal wall with the ectoascal wall still apparent.

DISCUSSION

The ascocarp in its youngest form is a mass of interwoven hyphae. These hyphae eventually coalesce and give rise to a pseudoparenchymatous structure which is divided into an outer layer of dark, thick-walled cells and a central zone of thin-walled cells. One of these centrally placed cells becomes differentiated into the ascogonium. The latter has thus arisen in a pre-formed stroma and the ascocarp is essentially an ascostroma. This fact, correlated with the bitunicate ascus, would place *Antennularia engleriana* in the subclass Loculoascomycetes (Luttrell, 1955).

The term, pseudoparaphyses, used here to describe the hypha-like structures which traverse the centrum from top to bottom is as defined by Luttrell (1965). As far as can be deduced from the sectioned material, these structures originate as outgrowths from the stromal cells especially those above the ascogonial complex. With subsequent downward growth, which may be mainly intercalary, they take on an almost vertical orientation and are attached at both ends. Their distinct, thread-like, branched and cellular make-up has been emphasized. According to Singer and Gamundi (1963) the term 'paraphysoid hyphae' has been ascribed to threads which are originally attached to the top of the ascocarp and which grow down but remain free from the bottom, and 'paraphysoid threads' for hyphal outgrowths attached at both top and bottom of the ascocarp from the beginning of development. Ontogenetic details of these two types do not strictly apply to the structures encountered in this fungus. Gäumann (1926) applied the term 'pseudoparaphysis' to the thread-like structures in the Loculoascomycetes, noting 'paraphysoid' (sensu Petrak, 1923) as a synonym. The objection against the use of 'pseudoparaphysis' is twofold. Firstly, it has been applied to an hymenial element in certain Basidiomycetes. Secondly, Petrak (1923) employed 'pseudoparaphysis' to describe hypha-like outgrowths which were free at the tips but these are better known as true paraphyses. The use of 'paraphysoid' has been avoided due to its application to both pseudoparaphysis and interthecial tissue. Further emphasis on the use of 'pseudoparaphysis' has been made by Luttrell (1965) who stresses these are the only distinctive structures in the Loculoascomycetes for which a special term is

warranted. The mode of origin and the development of the structures as found in this fungus corresponds with Luttrell's description and the terms 'pseudo-paraphysis' has been employed here.

The pseudoparaphysate centrum and the perithecium-like ascostroma places *A. engleriana* in the order Pleosporales (sensu Luttrell, 1955). Müller and von Arx (1950, 1962) have retained the order Pseudosphaeriales, established by Theissen and Sydow (1918), in which are included forms with uniloculate, perithecium-like ascostromata. The order Pleosporales has been used here instead of Pseudosphaeriales because of the indiscriminate inclusion in the latter of forms in which the centra contain either pseudoparaphyses or interthecial tissue. These structures, as indicated by developmental evidence, are not homologous.

It must be reiterated that the use of the generic name, *Antennularia* in preference to *Dimerosporiopsis* is tentatively agreed upon until other forms included in the former genus have been investigated. In fact, until investigations are carried out over a broader range among species grouped in the Pleosporales (of Luttrell) and the Pseudosphaeriales (of Müller and von Arx), can more definite criteria for subdivisions into various families be proposed.

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